

# Effect of Saponin Extracted from *Passiflora alata* Dryander (Passifloraceae) on development of the *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae)

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**Abstract** The saponins are glycosides with wide distribution among plants which may be toxic for herbivorous arthropods. Their insecticidal activity may be associated to the ability of producing alterations in the feeding behavior, in the molting process and causing death. This study evaluated the lethal and sub lethal effects of a saponins extract, obtained from *Passiflora alata* on *Spodoptera frugiperda*, by ingestion tests with artificial diet. Nine solutions with increasing extract concentration and a control solution with sterile distilled water were used in the test. In total 1500 insects were used in 5 repetitions, 30 replicates per concentration. Considering the total mortality, all treatments differed statistically from the control one, but not among them. Most of the observed effects were sub lethal, in which 68.3% insects presented deformation. The number of deformed insects per treatment increased as the extract of saponins concentration increased. In conclusion the mortality revealed significant difference between control and other treatments, which indicated the potential of saponins present in *P. alata* as a control agent of *S. frugiperda*. This potential needs to be better evaluated, especially if the facilities of raising and large scale production of the plant are considered.

**Keywords** Saponin, *Spodoptera frugiperda*, Lethal Effects, Fall Army worm, *Passiflora alata*

## 1. Introduction

The researches designed at identifying new toxic substances in order to control agricultural plagues are constant in the activities related to management and integrated control of plagues nowadays. This is partially a result of the knowledge of the negative impact of the chemical products used in the ecosystem as well as the possibility of evolution of the resistance of the target insects to the products used, whether these are chemical and/or biological. A great number of these researches are directed to the evaluation of toxicity of secondary metabolite of plants, such as phenols, alkaloids, glucosinolates, cyanogenic glycosides and saponins.<sup>1</sup> More than 2000 species of plants are known to possess some insecticidal

activity. In many cases plants have a history as traditional medicines or are used to kill or repel insects. In spite of the various studies available about the entomotoxic effects of extracts of different species of plants, their application is still incipient.<sup>2</sup>

The saponins are glycosides with detergent properties, due to the presence of hydrophobic (aglycones) and hydrophilic (sugar) components with wide distribution among plants which may be toxic for herbivorous arthropods such as mites, beetles and Lepidoptera, among others.<sup>3-7</sup> Their insecticidal activity may be associated to the ability of producing alterations in the feeding behaviour, in the molting process, of interacting with hormones that regulate the growth and causing death in the different stages of development.<sup>8-11</sup> Their wide spectrum of action reaches a great number of herbivores and its amplitude of physiological impacts makes these substances an excellent model for the study of the effect of natural substances with insecticidal activities.<sup>8</sup>

The passion fruit, *Passiflora alata*, is one of the main species of economic importance of the genus cultivated in

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Brazil, which is used mainly as fruit and for juice and sweets production as well as a medicinal plant.<sup>12-16</sup> The saponins are the main substances of its secondary metabolism. Five types of saponins were isolated and identified in its leaves: one is a steroid and four are triterpenic<sup>(3-6), 17-18</sup>.

The fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Noctuidae) is a polyphagous insect, using more than 50 species of plants as food supplies, distributed in over 20 families,<sup>19</sup> predominantly in grasses, among them corn and rice, which are important cultures for Brazilian and world economy. In rice, *S. frugiperda* is considered a pest of the initial phase, since it attacks the seedlings in the beginning of their development, feeding on the leaves and cutting the new stalk close to the ground,<sup>20</sup> causing losses of 14 to 24% of the grains. Depending on the population level, the destruction of the crop can be total.<sup>21</sup> In maize, the attack can occur from the seedling stage until the tasseling and the formation of spikes. In the later attacks specimens between the stalk and the cob can be found, where they penetrate the female inflorescence destroying the grain.<sup>22</sup>

The effect of saponins extract of some plants on the development of the *Spodoptera* species was demonstrated in studies with *S. litura*.<sup>2,4,23-27</sup> The results of these studies indicate alterations in the feeding behaviour, lengthening of the larvae and/or pupa stages, increased mortality and reduced fertility.

The study of plants with potential insecticidal activity is important to discover new active molecules, which may be isolated from plants or synthesized, or a molecule-prototype for structural changes to obtain a more active compound.

This work evaluated the effects of saponins extracts of *Passiflora alata* on *Spodoptera frugiperda*, identifying the sub lethal and lethal effects of these substances in different development stages of this species.

## 2. Material and Methods

### 2.1. Preparation and Characterization of the Saponin Extracts

To obtain saponin extract the dried leaves were submitted to maceration with 70% ethanol. The ethanol was filtered and placed in a rotary evaporator; the aqueous residue was extracted successively with chloroform, ethyl acetate and n-butanol. The evaporation of n-butanol fraction resulted in a fraction consisting mainly of saponins, which will be called henceforth saponin extract.<sup>17</sup> The saponin extract is characterized as a yellow-orange powder.

### 2.2. Obtention and Rearing of Insects

The establishment of *S. frugiperda* began with immature larvae collected in rice plantations, with the

collaboration of researchers at the Estação Experimental do Arroz (EEA) of the Instituto Riograndense do Arroz (IRGA), located in Cachoeirinha, RS. The insects were reared in the room for the creation of insects of the Laboratory of Microbiology of the University of Vale do Rio dos Sinos (UNISINOS), heated to 26°C, 75% RH and 12h photo phase. The adults were kept in plastic cages, fed with a glucose solution of 10%. Eggs were collected three times a week and put in gerbox with diet. Five or six days after hatching, the larvae were individualized in plastic cups, and maintained with artificial Poitout diet,<sup>28</sup> where they remained until they became pupae. As a routine procedure adopted, the pupae were identified, separated by sex, kept in sleeves with moistened filter paper and covered with tulle, until emergence of adults.

### 2.3. Bioassay

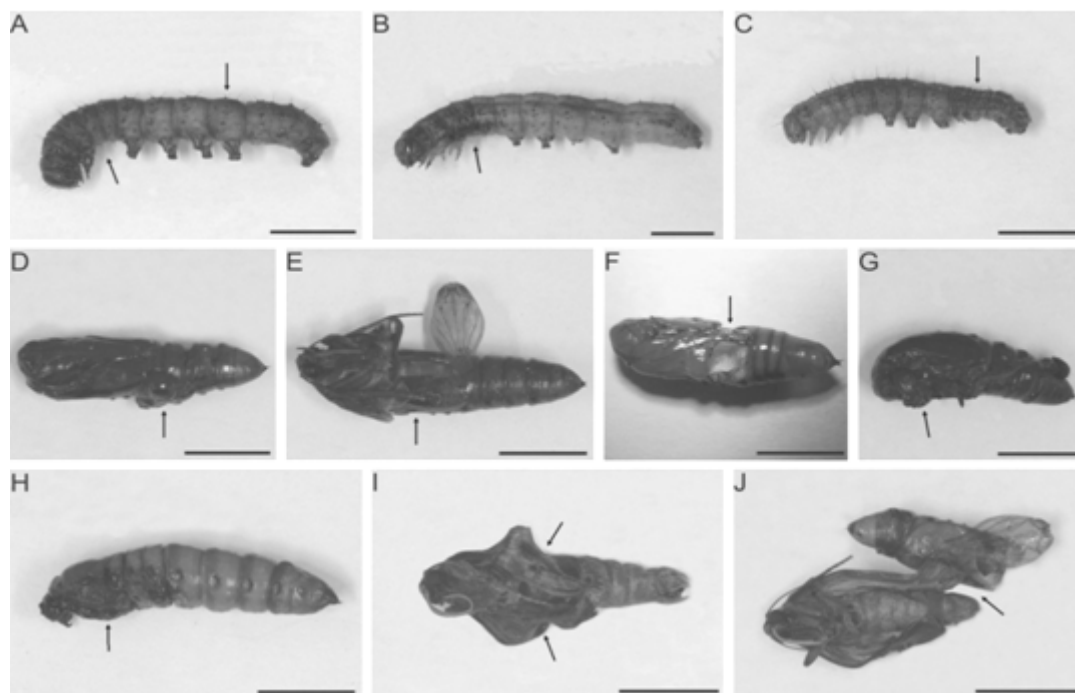
For the test with *S. frugiperda* nine solutions with increasing concentrations of saponin extract were used due to the 2x (312, 625, 1250, 2500, 5000, 10000, 20000, 40000 and 80000ppm) and a control of sterile distilled water. A solution of 20ml was prepared for each concentration, which, after use, was stored at 4°C. In this case, the extracts were diluted in sterile distilled water. The second instar larvae of *S. frugiperda* were maintained for seven days in mini acrylic plates (35mm diameter) containing Poitout diet, where 100µL of the solutions were applied on the diet. After this they were transferred to plastic cups containing only artificial diet, where they were observed until emergence of adults for the evaluation of possible sub lethal effects (Figure 1). Damages were evaluated under stereoscopic microscope and classified according to intensity (low, medium and high) (Figure 2).

Bioassays were kept at 26°C, 75% RH and 12h photo phase. Five repetitions of 30 larvae/concentration, totalizing 1500 insects were performed.

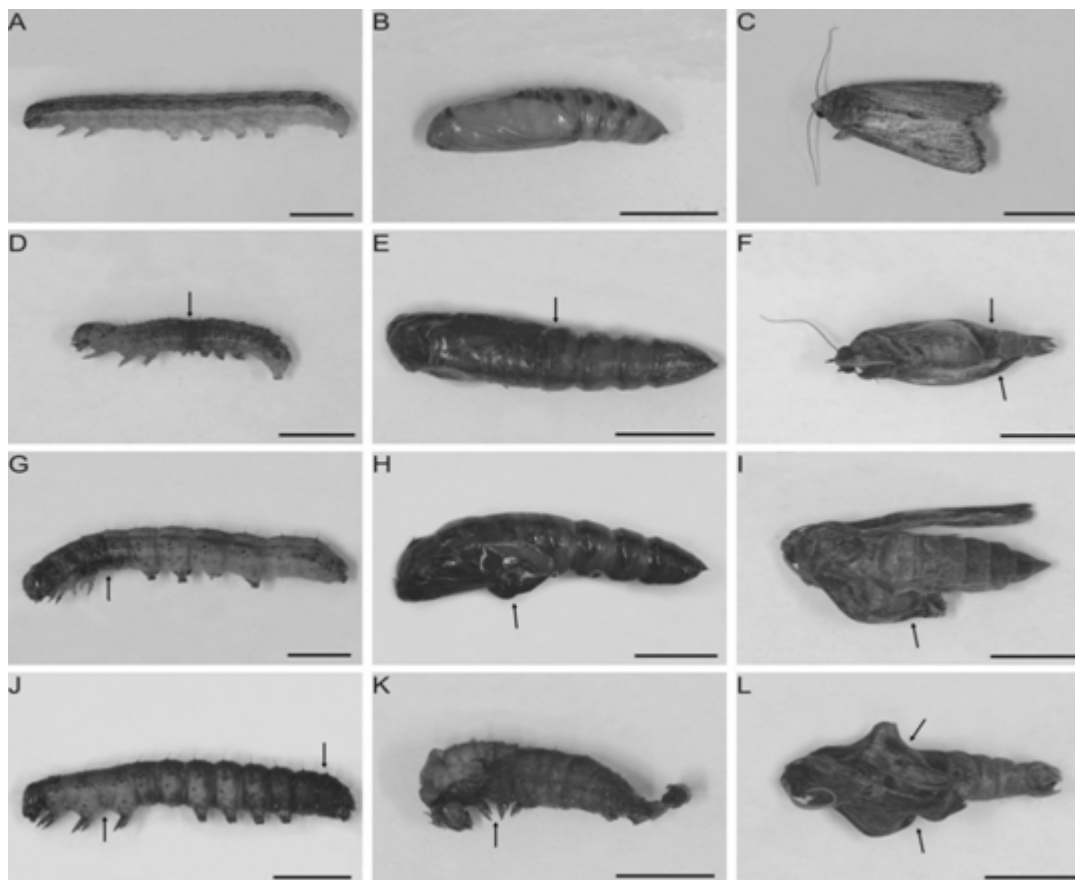
### 2.4. Data Analysis

Data were tested for normality using the chi-squared and Shapiro-Wilks and the homogeneity with the tests of Hartley and Bartlett. When these criteria were satisfied, they were compared by an ANOVA followed by multiple tests of Tukey. Otherwise, they were compared by Kruskal-Wallis followed by Dunn's multiple test. Statistical analysis was performed with the aid of TOXTAT Software version 3.3.<sup>29</sup> To compare mortality rates between larvae and pupae of *S. frugiperda* the Student t test was conducted for different means and to analyse the deformation of the same species a simple linear regression was made.<sup>30</sup>

## 3. Results



**Figure 1.** Characterization of the sublethal effects observed in *Spodoptera frugiperda*. A) Larva with abdominal distension; B) Larva with the point of necrosis in the integument; C) larva with incomplete molting process; D) Pupa with globular evagination in the integument; E) dead adults emerging from pupae; F) Pupa with failure in the process of sclerotization of the integument; G) Pupa with morphological features of larvae; H) Pupa with the point of necrosis in the integument; I) Adult with the wings unfolded e J) Adult with wings slightly distended and pupae attached on the wing



**Figure 2.** Characterization of the intensity of deformation in *Spodoptera frugiperda*. A) Larva without deformation; B) Pupa without deformation; C) Adult without deformation; D) Larva with deformation of low intensity; E) Pupa with deformation of low intensity; F) Adult with deformation of low intensity; G) Larva with deformation of medium intensity; H) Pupa with deformation of medium intensity; I) Adult with deformation of medium intensity; J) Larva with deformation of high intensity; L) Pupa with deformation of high intensity e M) Adult with deformation of high intensity

**Table 1.** Summary results of the experiments indicating mortality levels by treatments and stage of development

Treatments	Larvae Mortality		Pupae Mortality		Cumulative Mortality		Larval Period - days	Pupal Period - days
	total	%	total	%	total	%	mean	mean
Control	5	3	0	0	0	0	17.61	10.2
Treatment 1	58	37	26	17	84	56.0	29.18	10.69
Treatment 2	44	29	23	15	67	44.7	20.23	9.79
Treatment 3	59	39	25	17	84	56.0	26.41	9.92
Treatment 4	59	39	22	15	81	54.0	21.1	10.51
Treatment 5	54	36	39	26	93	62.0	25.59	8.87
Treatment 6	40	27	31	21	71	47.3	18.78	9.57
Treatment 7	48	32	39	26	87	58.0	20.19	9.48
Treatment 8	44	29	24	16	68	45.3	18.47	9.31
Treatment 9	30	30	39	26	69	46.0	18.78	9.75

**Table 2.** Summary results indicating the number of individuals with deformations by stage of development and treatments

	Single Deformations									Multiple Deformations								
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T1	T2	T3	T4	T5	T6	T7	T8	T9
Larvae	1	1	3	4	9	7	8	8	10	0	0	0	0	0	0	0	0	0
Pupae	2	5	4	4	6	3	4	6	1	1	1	0	0	2	6	9	2	1
Adults	20	32	32	25	36	24	30	29	42	0	0	1	0	1	10	2	2	3
Total	23	38	39	33	51	34	42	43	53	1	1	1	0	3	16	11	4	4

The quantitative and qualitative results of the experiments are indicated on tables 1 and 2, respectively

Considering the total mortality (larvae and pupae) all treatments differed statistically from control but not among them (Figure 3) ( $p < 0.05$ ). Comparing the mortality of larvae with the mortality of pupae in each treatment, it was observed that the rate of larval mortality was higher than that of the pupae from the control concentration until the concentration 2500ppm. At higher concentrations (5000 to 80000 ppm), these rates showed no significant difference (Figure 4). There were 441 of larvae and 268 of pupae deaths, i.e. 8.1% and 29.4% of organisms exposed to saponin, respectively, totalizing 17.9% of the insects used in bioassays.

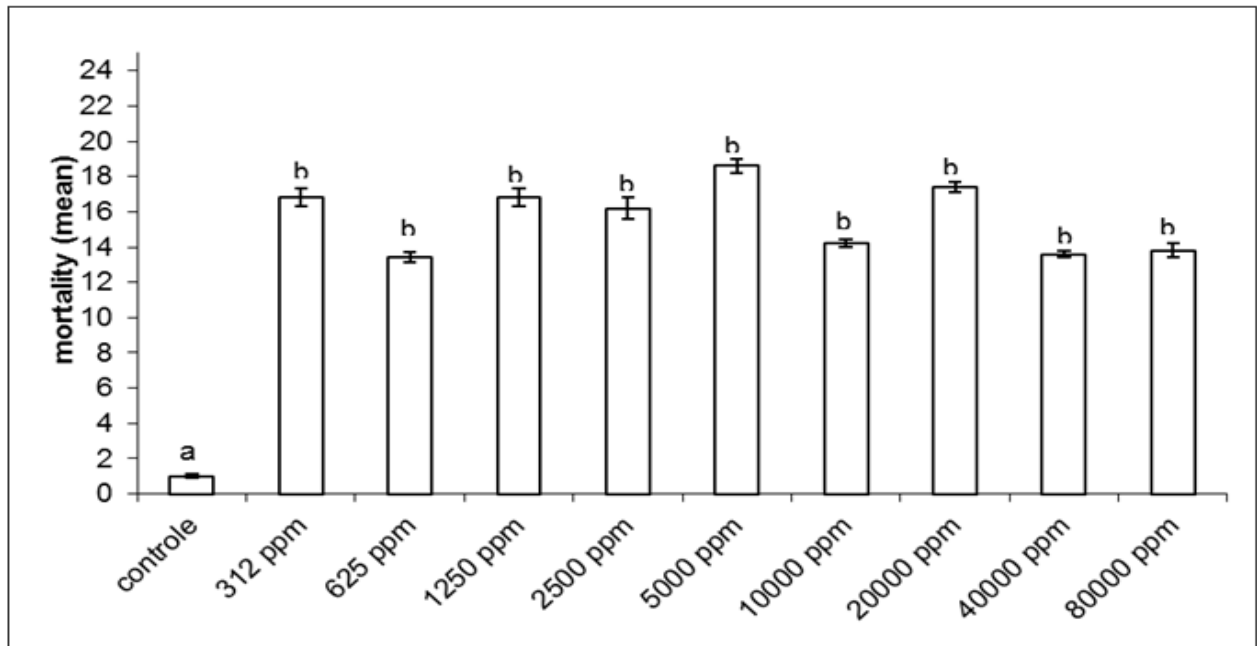
Of the 441 larva that died during the tests, 121 (27%) died with some deformity, of which 45 (37.2%) died during the process of molt, unable to release in whole or in part of molting, 8 (6.6%) had points of necrosis and 5 (4.1%) some parts of the body distended

Of the 268 dead pupae, 107 (39.9%) died with some deformity, of which 27 (25.2%) had retention of morphological characters of larvae, 88 (82.2%) died during the emergence of the adult failing to release in whole or in part, 5 (4.7%) had deformities in the form of globular

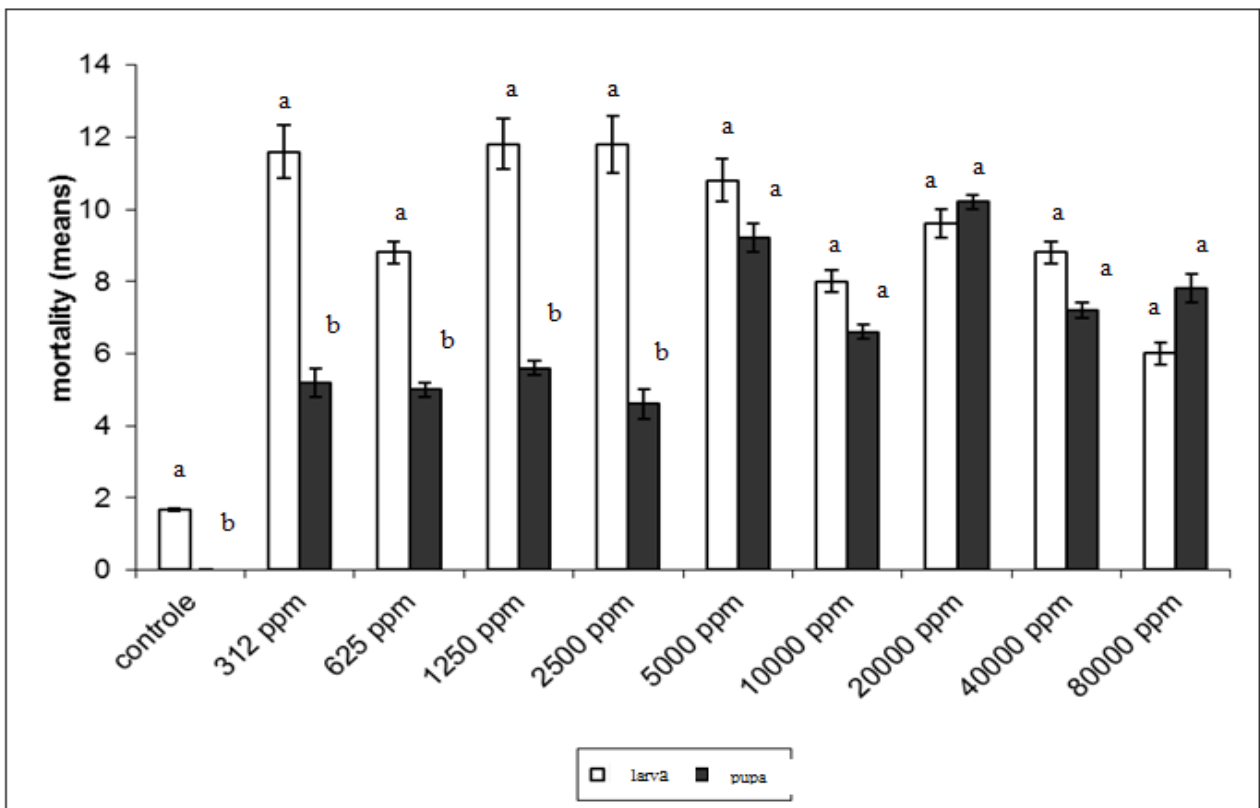
evaginations of the cuticle, 23 (21.5%) had points of necrosis in the cuticle and 11 (10.3%) sclerotization failures, leaving the internal organs exposed. Some specimens had more than one type of deformation, such as evaginations and points of necrosis (2.93%), or points of necrosis around the failures of the cuticle (0.97%). Often, when reviewing the experiments, the pupae with failure sclerotization were found still alive, being dead in 70% alcohol immediately to avoid the suffering of the individual. The main deformation observed in 599 adults obtained after the bioassays was in the region of the wings, where 200 (33.4%) insects showed little or no distension of the wings.

A total of 1025 insects (larvae, pupae and adults) were observed with deformations (76% of organisms) (Figure 5). These organisms were screened according to the intensity of their deformations: 612 (59.7 %) had deformities of low intensity, 144 (14.05%) of average intensity and 269 (26.2%) of high intensity (Table 3)

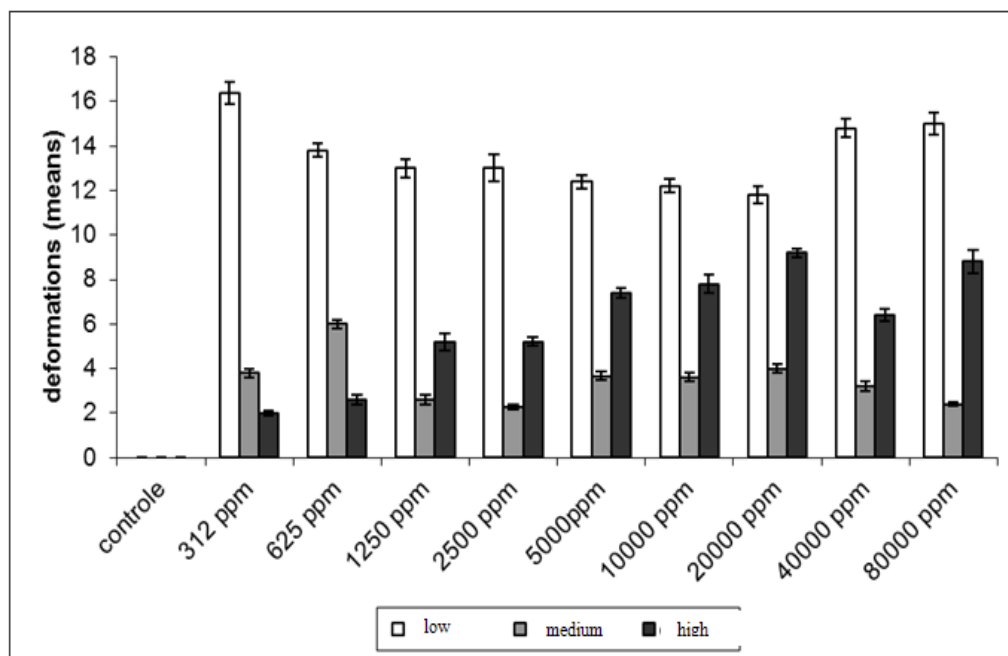
The linear regression obtained from the number of deformities observed in insects exposed to the saponin extracts, was a straight up line, which shows that there is a tendency to increase the number of deformed insects as the concentration of extract saponins increases (Figure 6)..



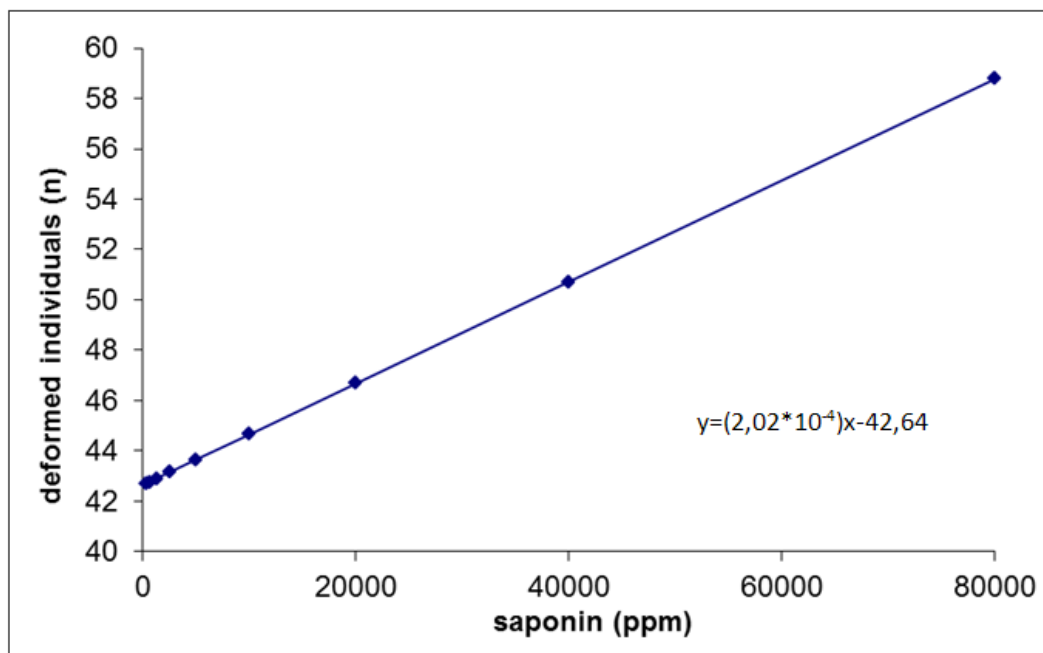
**Figure 3.** Total mortality (mean  $\pm$  standard error) of *Spodoptera frugiperda* after bioassay of ingestion in different concentrations of saponin. Columns followed by different letters are significantly different (ANOVA, followed by Tukey's multiple tests  $\alpha=0.05$ )



**Figure 4.** Mortality of larvae and pupae of *Spodoptera frugiperda* after ingestion test of saponin at different concentrations. Columns followed by different letters differ significantly at a given concentration (Student's t test,  $\alpha=0.05$ )



**Figure 5.** Deformations (mean  $\pm$  standard error) observed in *Spodoptera frugiperda*, after ingestion test of saponin, divided into three categories (low, medium and high), by concentration of saponin



**Figure 6.** Linear regression of the number of deformations observed in *Spodoptera frugiperda* after ingestion test of different concentrations of saponin

**Table 3.** Summary results of the damage levels by each treatment

Damage Level	T1	T2	T3	T4	T5	T6	T7	T8	T9
Low	82	69	65	65	62	61	59	74	75
Medium	19	20	13	9	11	18	16	16	12
High	8	9	26	24	37	39	46	32	44
Total	109	98	104	98	110	118	121	122	131

In summary the deformities were observed in 76% of organisms exposed to saponin. These deformations were

linked, primarily, to the process of molt, as 45 larvae of *S. frugiperda* died during molting process, failing to release in whole or in part of molted cuticle. Besides the problems related to the process of molt, the organisms that had points of necrosis in the larvae stage were located mainly in the thorax and the pupae stage and adults, in most cases, in the region of the wings. Moreover, problems were observed in sclerotization of pupae, which opened up large holes in the region of the wings, leaving the interior of organisms exposed. In organisms of control treatment no deformities were observed.

## 4. Discussion and Conclusions

This study identified the impact of saponins extract on the different stages of development of *S. frugiperda*. Besides the mortality of larvae and pupa, the presence of structural deformities was observed in all stages. When considered the total impact of the treatments 47.4% of the adults emerged without structural problems identified by optical microscopy. The effects of the saponin extract on *S. frugiperda* may be associated to a deterrent toxic action and/or by its interactions with substances responsible for the different stages of development of the specie.

The deterrent effect reduces the consumption of food producing nutritive deficiency causing deficient growth or deformities, which may lead to death or inhibit the progress for the next stage.<sup>26,27,31,32</sup> Among the alterations observed, the presence of necrosis points stood out, sclerotization problems and retention of larvae characters and tumors. These results are similar to the ones registered,<sup>21</sup> for the biotypes of corn and rice of *S. frugiperda* using different extracts of different species of plants. It is important to highlight that alterations in the length of the larvae and pupal stages were not observed in this study, as registered for *S. littoralis*<sup>23</sup> e for *S. frugiperda*.<sup>33</sup> The differences in these results may be associated to the variation in the composition of saponins among the plant species used for the acquisition of the extracts.<sup>3,34</sup>

The physiological basis of the toxicity of saponins has not been fully elucidated, but we know that there is a great interaction between them and the cell membranes, with effects on the hydrophobic-lipophilic balance and permeability of these because they are capable of forming complexes such as sterols, for instance, cholesterol.<sup>10,11,35</sup> The toxicity of saponins on arthropods may derive from their ability of interacting with free steroids of the intestine and/or of inhibiting the digestive proteases, reducing the rates of digestion and absorption.<sup>8,35,36</sup>

The mortality and deterrence caused by saponin were also observed in experiments that evaluated the effects of different types of saponin for aphids,<sup>38,39,42</sup> nematodes,<sup>40-42</sup> beetles<sup>31,43,44</sup> and *Spodoptera* sp.<sup>2,5,6,25,27</sup>

A fact that drew attention was the appearance of specimens in the intermediary period between larvae and pupae (1.8% of exposed organisms). Apparently, the organisms began the process of becoming pupae and could not complete it, which led to death. The appearance of intermediate individuals between pre-pupae and pupae can occur when the activity of juvenile hormone, which controls the metamorphosis, is affected.<sup>32</sup>

Furthermore, the metamorphosis may have been affected because of saponins form complexes with sterols, like cholesterol, both in the intestine in cell membranes of insects. These complexes formed by saponin make sterols unavailable, influencing the synthesis of ecdison, one of the hormones involved in the process of molt. It has been shown that insects are unable to produce these sterols,

removing them entirely from the diet.<sup>10,11,34,36,39</sup> The statements of these authors may account for the large number of deaths of larvae and pupae during the processes of molt and emergence of pupae malformed, beyond the failures of sclerotization observed.

In conclusion the mortality of *S. frugiperda* showed significant difference between control and other treatments. It was observed that the death of the specimens was not immediate after the ingestion of saponin, but slow and gradual, which apparently occurred under the influence of saponin in the metabolism of the larvae, pupae and adults. These results indicate the potential of saponins present in *P. alata* as a control agent of *S. frugiperda*. This potential needs to be better evaluated, especially if the facility of raising and large scale production of the plant is considered.

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## REFERENCES

- [1] Tagliari MS, Knaak N and Fiuza LM. Plantas inseticidas: interações e compostos. Pesquisa Agropecuária Gaúcha 10: 101-111, 2004.
- [2] Ulrichs C, Mewis I, Adhikary S, Bhattacharyya A and Goswami A. Antifeedant activity and toxicity of leaf extracts from *Porteresia coarctata* Takeoka and their effects on the physiology of *Spodoptera litura* (F.). J Pest Sci 81: 79-84, 2008.
- [3] Vincken JP, Heng L, de Groot A and Gruppen, H. Saponins, classification and occurrence in the plant kingdom. Phytochemistry 68: 275-297, 2007.
- [4] Kamaraj C, Rahuman AA and Bagavan A. Antifeedant and larvicidal effects of plant extracts against *Spodoptera litura* (F.), *Aedes aegypti* L. and *Culex quinquefasciatus* Say. Parasitol Res 103: 325-331, 2008.
- [5] Park HY, Kim JY, Kim HJ, Lee GH, Park JI, Lim CW, Kim YK and Yoon HD. Insecticidal and Repellent Activities of Crude Saponin from the Starfish *Asterias amurensis*. J Fish Sci Technol 12:1-5, 2009.
- [6] Saha S, walia S, Kumar J, Dhingra S and Parmar BS. Screening for Feeding Deterrent and Insect Growth Regulatory Activity of Triterpenic Saponins from *Diplonema butyracea* and *Sapindus mukorossi*. J Agric Food Chem 58: 434-440, 2010.
- [7] Prasifka JR, Bradshaw JD, Lee ST and Gray ME. Relative Feeding and Development of Armyworm on Switchgrass and Corn, and Its Potential Effects on Switchgrass Grown for Biomass. J Econ Entomol 104:1561-1567, 2011.
- [8] IKBA I. Saponins as insecticides: a review. Tunisian Journal of Plant Protection 5: 39-50, 2010.

- [9] De Geyter, E, Lambert E, Geelen D and Smagghe G. Novel advances with plant saponins as natural insecticides to control pest insects. *Pest Technol* 1: 96-105, 2007.
- [10] De Geyter E, Smagghe G, Rahbé Y and Geelen D. Triterpenesaponins of *Quillajasaponaria* show strong aphicidal and deterrent activity against the pea aphid *Acyrtosiphon pisum*. *Pest ManagSci* DOI:10.1002/ps.2235, 2011.
- [11] De Geyter E, Swevers L, Soin T, Geelen D and Smagghe G. Saponins do not affect the ecdysteroid receptor complex but cause membrane permeation in insect culture cell lines. *J insect physiol* 58:18-23, 2012.
- [12] Cervi AC. O gênero *Passiflora* L. (Passifloraceae) no Brasil, espécies descritas após o ano de 1950. *Adumbrationes ad Summae Editionem* 16: 1-5, 2006.
- [13] Reginatto FH, De-Paris F, Petry RD, Quevedo J, González Ortega G, Gosmann G and Schenkel EP. Evaluation of anxiolytic activity of spray dried powders of two south Brazilian *Passiflora* species. *Phytother Res* 20: 348-51, 2006.
- [14] Mello FB, Langeloh A and Mello JRB. Toxicidade Pré-Clinica de Fitoterápico Contendo *Passiflora alata*, *Erythrina mulungu*, *Leptolobium elegans* e *Adonisvernalis*. *Lat Am J Pharm* 26:191-200, 2007.
- [15] Barbosa PR, Valvassori SS, Bordignon CL, Kappel VD, Martins MR, Gavioli EC, Quevedo J and Reginatto FH. The aqueous extracts of *Passiflora alata* and *Passiflora edulis* reduce anxiety-related behaviors without affecting memory process in rats. *J Med Food* 11: 282-8, 2008.
- [16] Gosmann G, Provensi G, Comunello LN and Rates SMK. Composição química e aspectos farmacológicos de espécies de *Passiflora* L. (Passifloraceae). *Rev Bras Biocien* 9: 88-99, 2011.
- [17] Reginatto FH, Kauffmann C, Schripsema J, Guillaume D, Gosmann G and Schenkel EP. Steroidal and Triterpenoidal Glucosides from *Passiflora alata*. *J Braz Chem Soc* 12: 32-36, 2001.
- [18] Reginatto FH, Gosmann G, Schripsema J and Schenkel EP. HPLC/UV Assay of quadrangulose, the major saponin from *Passiflora alata* leaves. *Phytochemical Analysis* 15: 195-197, 2004.
- [19] Cruz I. A lagarta do cartucho do milho. Circular técnica 21, EMBRAPA/CNPMS. (EMBRAPA/CNPMS, Sete lagoas, pp 1-45 (1995).
- [20] SOSBAI. Arroz Irrigado. Recomendações técnicas da pesquisa para o sul do Brasil. SOSBAI/UFMS/IRGA/EPAGRI, Santa Maria, RS 159p., 2005.
- [21] Busato GR, Garcia MS, Loeck AE, Zart M, Nunes AM, Bernardi O and Andersson FS. Adequação de uma dieta artificial para os biótopos do “milho” e “arroz” de *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Bragantia* 65: 317-323 (2006).
- [22] Oliveira MSS, Roel AR, Arruda EJ and Marques AS. Eficiência de produtos vegetais no controle da lagarta-do-cartucho-do-milho *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). *Ciência e A grrotecnologia* 31: 326-331, 2007.
- [23] Adel MM, Sehnael F and Jurzysta M. Effects of alfalfa saponins on the moth *Spodopteralittoralis*. *J ChemEcol* 26: 1065-1078, 2000.
- [24] Ikbal C, Monia BHK, Mounir T, Wassila H, Najet R, Dorsaf BA, Mejda D and Habib BHM. Toxicity investigation of *Cestrum parqui* saponins to *Culex pipiens* larvae. *J BiolSci* 4:113-120, 2007.
- [25] Ikbal C, Mounir T, Mounia BHK and Habib BHM. Histological Effects of *Cestrum parqui* Saponins on *Schistocerca gregaria* and *Spodopteralittoralis*. *J BiolSci* 7: 95-101, 2007.
- [26] Zapata N, Budia F, Vinuela E and Medina P. Antifeedant and growth inhibitory effects of extracts and drimanes of *Drimys winteri* stem bark against *Spodopteralittoralis* (Lep.Noctuidae). *Ind Crop Prod* 30: 119-125, 2009.
- [27] Pavela R. Antifeedant activity of plant extracts on *Leptinotarsa decemlineata* Say. and *Spodopteralittoralis* Bois Larvae. *Ind Crop Prod* 32: 213-219, 2010.
- [28] Pouitout S and Bues R. Élevage de plusieurs espèces de Lépidoptères Noctuidae sur milieu artificiel riche et sur milieu simplifié. *Ann Ecol Anim* 2: 79-91, 1970.
- [29] Gulley DD, Boelter AM and Bergman HL. TOXTAT 3.3 Computer Program, 1994.
- [30] Callegari-Jacques SM. Bioestatística: princípios e aplicações. Artmed 255p., 2003.
- [31] Gazzoni DL, Hülsmeier A and Hoffmann-Campo CB. Efeito de diferentes doses de rutina e quercetina na biologia de *Anticarsia gemmatilis* Hübner, 1818 (Lep., Noctuidae). *Pesq Agropec Bras* 32: 673-681, 1997.
- [32] Costa ELN, da Silva RFP and Fiuza L.M. Efeitos, aplicações e limitações de extratos de plantas inseticidas. *Acta Biologica Leopoldensia* 26: 173-185, 2004.
- [33] Augustin JM, Kuzina V, Andersen SB and Bak S. Molecular activities, biosynthesis and evolution of triterpenoid saponins. *Phytochemistry* 72: 435-457, 2011.
- [34] Rosenthal GA and Berenbaum MR. Herbivores – their interactions with secondary plant metabolites. Vol 1 Academic Press, New York 468p, 1991.
- [35] Gershenzon J and Croteau R. Terpenoids. pp.165-219. In: Rosenthal, G.A. & Berenbaum, M.R. (eds.) *Herbivores: their interactions with secondary plant metabolites*. Academic Press, New York, 468p, 1991.
- [36] Sylwia G, Bogumil L. and Wieslaw O. Effect of low and high- saponin lines of alfalfa on pea aphid. *J Insect Physiol* 52: 737-743, 2006.
- [37] Golawska S. Deterrence and Toxicity of Plant Saponins for the Pea Aphid *Acyrtosiphon pisum* Harris. *J ChemEcol* 33:1598-1606, 2007.
- [38] Pelah D, Abramovich Z, Markus A and Wiesman Z. The use of commercial saponin from, *Quillajasaponaria* bark as a natural larvicidal agent against *Aedes aegypti* and *Culex pipiens*. *J Ethnopharmacol* 81: 407-409, 2002.
- [39] Santiago GMP, Viana FA, Pessoa ODL, Santos RP, Pouliquen YBM, Arriaga AMC, Andrade-Neto M and Braz-Filho R. Avaliação da atividade larvicida de saponinas



- triterpênicas isoladas de *Pentaclethra macroloba* (Willd.) Kuntze (Fabaceae) e *Cordia alliodora* Fresen (Boraginaceae) sobre *Aedes aegypti*. *Rev Bras Farmacogn* 15: 187-190, 2005.
- [40] Kumar MS and Maneemagalai S. Evaluation of Larvicidal Effect of *Lantana camara* Linn Against Mosquito Species *Aedes aegypti* and *Culex quinquefasciatus*. *Adv Bio Res* 2: 39-43, 2008.
- [41] Bagavan A, Rahuman AA, Kamaraj C and Kannappan G. Larvicidal activity of saponin from *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 103:223-229, 2008.
- [42] Waligóra D. Activity of the saponin extract from the bark of *Quillaja saponaria* Molina, against colorado potato beetle (*Leptinotarsa decemlineata* Say). *J Plant Protec Res* 46:199-206, 2006.
- [43] Nielsen JK, Nagao T, Okabe H and Shinoda T. Resistance in the Plant, *Barbarea vulgaris*, and Counter-Adaptations in Flea Beetles Mediated by Saponins. *J Chem Ecol* 36: 277-285, 2010.